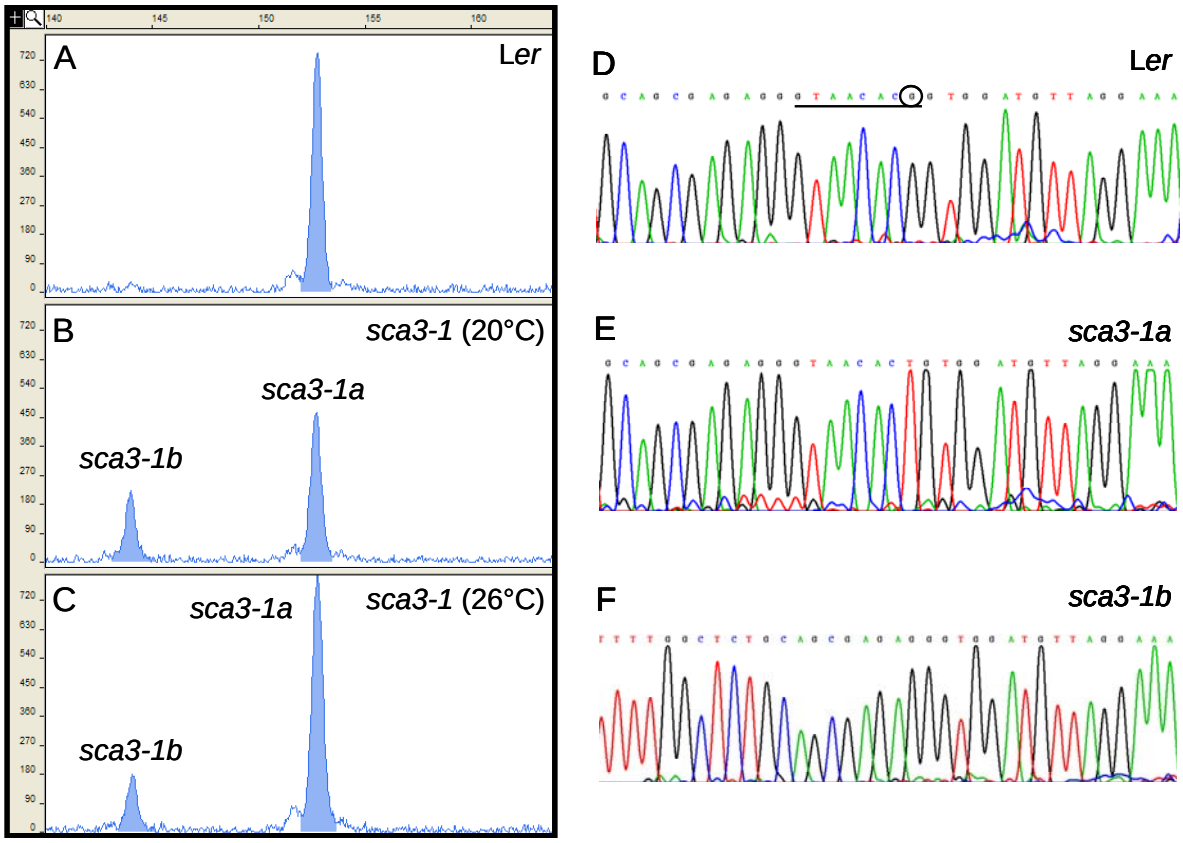
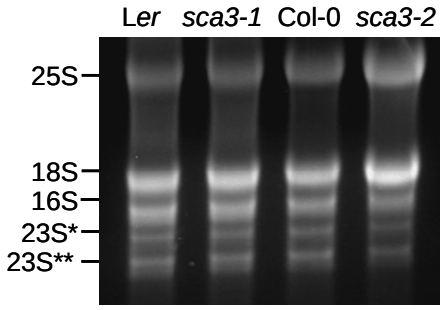
SUPPLEMENTAL DATA

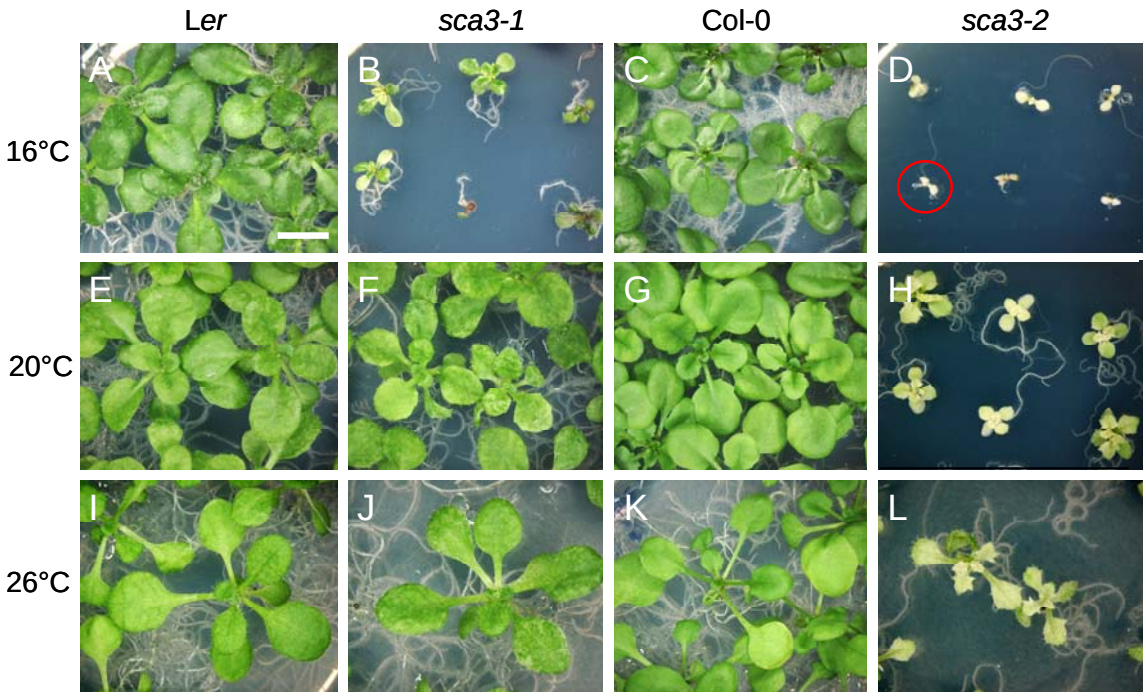


Supplemental Figure 1. (A-C) Fragment analysis electropherograms illustrating fluorescence-based detection of *SCA3* cDNAs, performed following the method described in Ponce et al. (2000). Total RNA was extracted from leaves of the L*er* wild-type (A), and the *sca3-1* mutant (B, C) grown at 20°C (B) and 26°C (C). Two *SCA3* splice forms are present in the mutant, whose lengths differ in 8 nt. The horizontal and vertical axes indicate, respectively, the size of the electrophoresed molecules (in nucleotides) and the intensity of fluorophore emissions (in arbitrary units of fluorescent signal strength). The electropherograms were produced by the Genescan 3.7 software and had been simplified by removing the peaks corresponding to the internal molecular weight standard. Every peak is denoted with the name of the mRNA to which it corresponds. (D-F) Sequencing electropherograms showing the 16th-17th exon junction region of the *SCA3* gene in cDNA obtained from the wild-type L*er* (D), and the *sca3-1a* (E) and *sca3-1b* (F) splice forms found in the *sca3-1* mutant. The F10 forward primer and the sca3-1aR and sca3-1bR reverse primers (Supplemental Table 1) allowed the separate amplification of the *sca3-1a* and *sca3-1b* cDNAs, which were sequenced using the F10 primer. A circle indicates the nucleotide change found in *sca3-1a.* The 8-nt segment absent from *sca3-1b* mRNA is underlined.

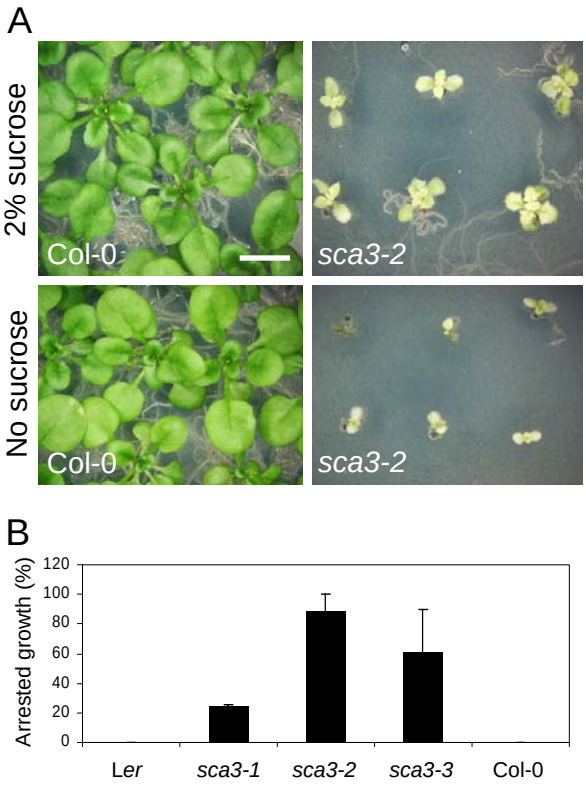


25S	1.0	1.0	1.0	1.0
16S	1.2	1.2	1.2	0.5
23S*	0.7	0.7	0.7	0.3
23S**	0.9	0.8	0.9	0.3

Supplemental Figure 2. rRNA abundance in the *sca3* mutants. Total RNA was extracted from 3-week-old wild-type (L*er* and Col-0), and *sca3-1/sca3-1* and *sca3-2/sca3-2* mutant plants. Eight μg from each line were separated on a denaturing formaldehyde gel and visualized by ethidium bromide staining. The asterisks denote the *in vivo* breakdown product of chloroplast 23S rRNA. Numbers at the bottom indicate the levels of chloroplast 16S, 23S* and 23S** rRNAs relative to those of the cytoplasmic 25S rRNA in a given sample. rRNA quantification was performed using the Image-J program (http://rsb.info.nih.gov/ij/docs/menus/file.html).



Supplemental Figure 3. Cold sensitivity of the *sca3* mutants. Plants were grown at the temperatures shown. A circle highlights a *sca3-2/sca3-2* seedling grown at 16°C and arrested in the stage of expanded cotyledons, a phenotype never observed in the wild types. All plants were homozygous for the mutations shown. Pictures were taken 21 days after sowing. The scale bar indicates 5 mm.



Supplemental Figure 4. Effect of sucrose on the growth of the *sca3-2* mutant. (A) Nineteen-day-old plants grown on culture media in the presence or absence of sucrose. All plants were homozygous for the mutations shown. The scale bar indicates 5 mm. (B) Percentage of plants with arrested development caused by the absence of sucrose. Data are means of two different replicates of 50-100 seeds, scored three weeks after sowing. We considered as arrested those seedlings displaying expanded cotyledons and a first pair of leaves of small size.

Supplemental Table 1. Primers used in this work

Purpose	Primer names (Forward/Reverse)	Oligonucleotide sequences (5´→3´)	
		Forward primer	Reverse primer
RT-PCR and cycle sequencing	At2g24120F1/R1	GGGATATTTAATGTTCATATTCC	CACGACTCAACACATCATTTC
	At2g24120F2/R2	GCTCCTCATATTGAGCTTTTG	CACAGCATTTGCATACGTAAG
	At2g24120F3/R3	CAGGCTAAGCATATGTTAATTC	GAATCCATTATATCATCCAAATG
	At2g24120F4/R4	CGTGGAACCCTGGAGTTTC	ACCATATACTGACGTCATTAC
	At2g24120F5/R5	TGTCAGGTGTAGCTTCTTATG	GCAGTTTAGTTCTGTCAATTC
	At2g24120F6/R6	GCAGTTTAGTTCTGTCAATTC	ATCAGTTTGTCTTTCTTGCAC
	At2g24120F7/R7	TGAAACATATGTTCTTGGGATG	TAAACCTGTGTCTGAATGCAG
	At2g24120F8/R8	CCTCTATGCAGGTGGTGTTG	GATAAGGATCTTTGCCAGTGC
	At2g24120F9[a]/R10	TGCAGAAGTGAAAGACATCTG	AACAGCAGTCATCATCATGTG
	At2g24120F10	GCTGCTGCTTGCTATTCTGCA	
	sca3-1aR		TTCCTAACATCCACAGTGTTAC
	sca3-1bR		TTCCTAACATCCACCCTCTCG
	OTC3D/R	TCCTTGCCAAATCATGGCCG	GCATGCATGCGATTCTCCGC
Confirmation of T-DNA inserts	LBa1	TGGTTCACGTAGTGGGCCATCG	
	LBb1	GCGTGGACCGCTTGCTGCAACT	
	RpoTmpF1/R1[b]	TCTCATATGATGATGACTGC	CATTACTTTCTTTATCAGTT
	BastaF1/R1[b]	GTCCAGCTGCCAGAAACCCACGTCATG	CCATCGTCAACCACTACATCGAGACAAG
qRT-PCR	PE-At1g68990F/R	GGAGCCAGTATATGAGGCTTTA	CTCTTCTGGAATGGGTACATCTT
	PE-At5g15700F/R	GGTAGCGAAAGGAAGCATGAAT	GCTTGGCTCCATGAGTTTTCAT
	PE-At2g24120F/R	CTTGGTGATTGTGCAAAGATAATT	GGGAGGAAATGCAGTTCTTTGTT
	PE-ArthCp048F/R	GGTTGACATATACAACCGACTTT	CCATCCACCAGGAGAGTTTATA
	PE-ArthCp044F/R	GACGGGTGAATAGAGTGACTTT	GGAGTCGACTCACTTCTTTCAA
	PE-ArthCp031F/R	GCTAAGTAAAGCAATGGATAGTTT	CGAATGTCCTTGGAGCTAACTAA
	PE-Atcg00190F/R	GCAGGTTAGAATTAGAGATTGATA	GGGTAGCAAACATTCTCTAGAAT
	PE-ArthCp013F/R	GCCTAGTATACTGCGATTTTTC	CTCGATTTCGAAATATATCGAAAC
	PE-At1g24260F/R	TTAGCAGTTGAACTTAGTAGCC	CCAAGATCTTCTCCCAACAGAT
	PE-At1g30380F/R	CCTGTACAAGGCCTGGCAA	CTGATGGCGCAAGTCCGAAT
	PE-At1g64860F/R	CACACCCTCCATTGATAGGATT	CCAGGGAGACCATTCAAAGAA
	PE-At2g05070F/R	CTCCCCAAAGCATCTGGTAT	CCCATCTGCTGTGGATTACTT
	PE-At5g67030F/R	GGCATTTGGTCTAAGGTGAGAA	CAGACTCGATATCCGCTGGTA
	PE-OTCF/R	TGAAGGGACAAAGGTTGTGTA	CGCAGACAAAGTGGAATGGA

[a]Primer labeled with 6-FAM phosphoramidite. [b]Primers used to confirm the presence of a T-DNA insertion in the *rpoT;2* mutant.

Supplemental Table 2. Chlorophyll and carotenoid content in the *sca3* mutants

Genotype	Chlorophyll a	Chlorophyll b	Carotenoids
Col-0	999.40 ± 122.05	421.18 ± 130.24	288.28 ± 77.34
sca3-2/sca3-2	193.32 ± 70.86	39.70 ± 12.48	93.74 ± 23.91
L*er*	993.91 ± 122.14	482.22 ± 140.67	256.03 ± 35.17
sca3-1/sca3-1	612.34 ± 148.80	236.95 ± 99.94	215.12 ± 43.31

Chlorophylls and carotenoids were extracted as described in Methods. Values represent means ± standard deviations (in micrograms per gram of fresh weight) of four independent samples, each containing 80 mg of 3-week-old plants. All the mutant values were significantly different ($P < 0.05$) from those of the corresponding wild type (Col-0 for *sca3-3* and L*er* for *sca3-1*), the only exception being the carotenoid content of *sca3-1/sca3-1* plants.